Influenza virus infection in quail (Coturnix coturnix): characterization of humoral immune response

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Introduction and Objective

Quail (*Coturnix coturnix*) has been suggested as a potential intermediate host in the interspecies transmission of influenza viruses. Similarly to pigs, quail is potentially susceptible to infection with both avian and human influenza viruses (1, 2). Then, since quail have emerged as a potential spreader of avian influenza viruses (AIV), new efforts have been made to clarify the epidemiologic role of this species in avian influenza infection. This study sought to better understand the humoral immune response generated by different influenza virus in quail.

The objective of the present work was to study the humoral immune response generated by a low pathogenic avian influenza virus (LPAIV) H5N2 and a human H1N1 (pH1N1) virus infection in quail.

Materials and Methods

Forty-eight quails were divided into three groups housed in independent isolation units at the biosafety level 3 (BSL-3) facilities of CReSA. Birds on group 1 (G1; n=20) were inoculated by oculonasal route with H5N2 LPAIV (10^6EID_{50}/50µL), birds on group 2 (G2; n=20) were inoculated with pH1N1 (10^6EID_{50}/50µL) and birds on group 3 (G3; n=8) were inoculated with saline solution; thus served as a negative control. Five animals of each infected group and two animals of the control group were euthanized at 3, 6, 10 and 12 days post-inoculation (dpi). Blood, choanal, tracheal and cloacal swab samples were collected. Blood was used to evaluate seroconversion and antibodies isotype dynamics by competition ELISA (cELISA). Swabs were used to study viral shedding by quantitative RT-PCR. Direct ELISA tests were performed in order to determine if quail antibodies could be recognized by commercial antibodies against chicken antibodies.

Results and Discussion

Quail supported the replication of H5N2 subtype (data not shown) and seroconversion was present in G1 group (H5N2 LPAIV) (Figure 1). On the contrary, only one bird belonging to G2 group (pH1N1) showed viral shedding on both choanal and tracheal swabs at 3 dpi (data not shown) and they did not show seroconversion (Figure 1). Anti-chicken goat IgG polyclonal antibodies (Bethyl Laboratories, INC., Montgomery, USA) recognized IgY and IgM quail antibodies in serum (Figure 2). Quail IgM isotype antibodies against influenza nucleoprotein (NP) were found in serum samples from H5N2 infected quails with a strong humoral immune response (Figura 3). Most of the animals inoculated with H5N2 LPAIV were capable to develop a humoral immune response in front of the infection. These data correlate well with the replication capability of the viruses tested. We demonstrated that anti-IgY and IgM chicken antibodies cross-reacted with quail antibodies and they can be used for serological studies in quails. In H5N2 infected animals, IgM isotype antibodies could be detected as early as 6 dpi and a poor response by IgY isotype antibodies was detected at that time, indicating that the days sampled in this experimental infection were too early to detect them effectively.

Conclusion

No available information has been found regarding immunoglobulin isotypes dynamics in systemic immune responses to Influenza infection in avian species. Therefore, this is the first time that antibodies of the IgM and IgY isotypes are characterized in an Influenza infection in quail. Moreover, commercial reagents are limited and generally ready for chicken samples. In this study, we demonstrated that anti-IgY and IgM chicken antibodies cross-reacted with quail antibodies. Altogether, these results will be very useful for further studies with this avian species.

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Figure 1. Evolution of the inhibition percentage mean along the experimental procedure for each group. Competition Enzyme Linked Immunosorbent Assay (ELISA) for the detection of anti-NP antibodies. Standard deviation within the group is given by vertical bars. Titers ≤ 60% were considered positive.

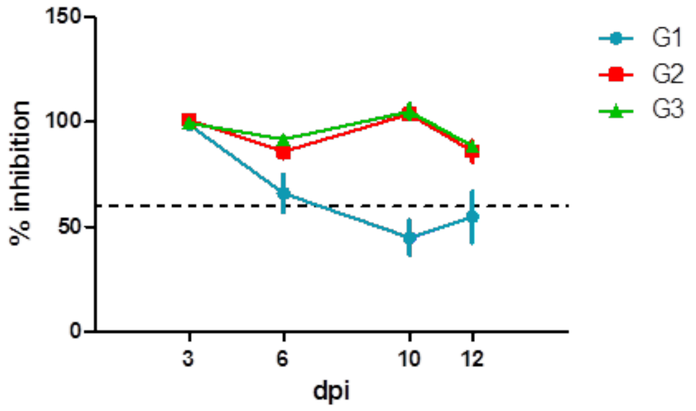


Figure 2. Direct Enzime Linked Immunosorbent Assay (ELISA) for IgY and IgM isotype antibodies detection. A) In chicken serum. **B)** In quail serum. Results are expressed as OD-value and shown as means ± SD. OD, optical density.

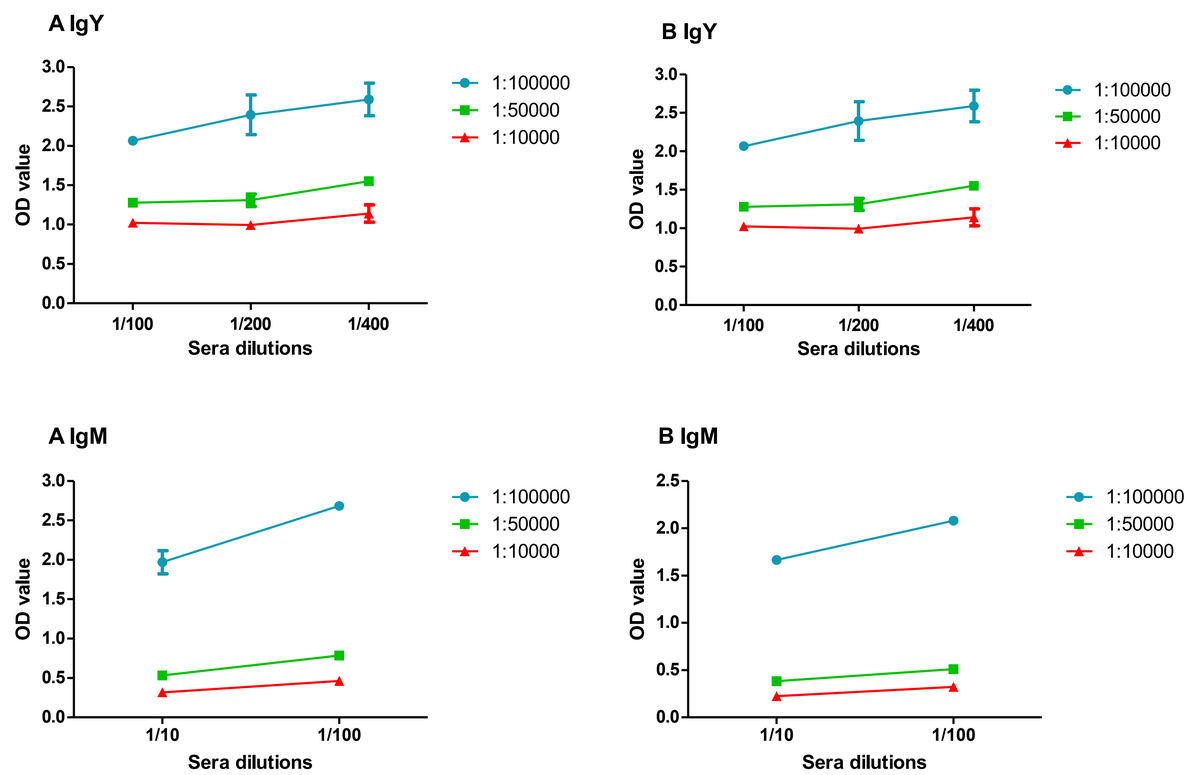
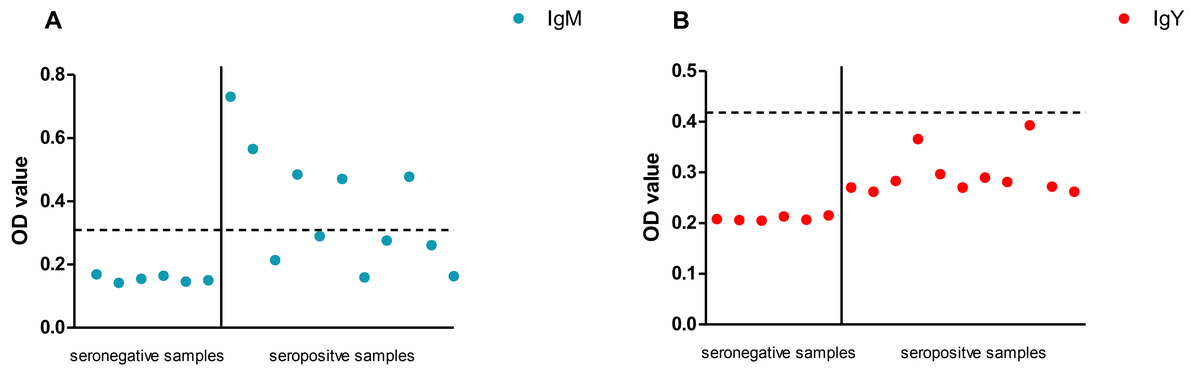


Figure 3. Nucleoprotein (NP) specific IgM and IgY antibodies in serum samples. Seronegative animals are located in left side of the vertical line whereas seropositive animals are in the right side. The discontinued horizontal line represent the positivity threshold. Results are expressed as OD-value and shown as means. OD, optical density.



References

1. Makarova NV, Ozaki H, Kida H, Webster RG *et al*. **Replication and transmission of influenza viruses in Japanese quail**. *Virology* 2002, **310:**8-15.

2. Thontiravong A, Kitikoon P, Wannaratana S, Tantilertcharoen R, et al. **Quail as a potential mixing vessel for the generation of new reassortant influenza A viruses**. *Vet Microbiol* 2012, **160(3-4):** 305-13.